# Mentype® **DIPscreen**
## Manual

**The entry to a quantitative chimerism analysis**

Biotype® Diagnostic GmbH

In-Vitro-Diagnostics

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Made in Germany

Biotype Diagnostic GmbH develops, produces and markets their PCR-based rapid Mentype® Detection Kits. Our products provide customers with fast and reliable testing methods for professional medical diagnostics.

Our Mentype® Test Kits guarantee highest quality standards for clinical research and diagnostics.

For information and enquiries about the Mentype® **DIPscreen** please do not hesitate to get in touch or visit www.biotype.de/en/home.html.

## Product description

Mentype® **DIPscreen** mediates the switch from multiplex-PCR approaches towards the highly quantitative real-time PCR technique to monitor chimerism. The flexible assay format allows an individual diagnostics at any time required.

Analysis of molecular chimerism resulting from allogeneic stem cell transplantation has become a well established method to control the course of transplant engraftment and to assess the risk of threatening relapse. Molecular chimerism analysis can be performed on diverse DNA-sequence motifs of which biallelic short insertion/deletion polymorphisms (DIPs, INDELs) offer substantial benefits. Polymerase-mediated amplification of DIP-markers does not result in formation of stutter peaks that can hamper clear analysis. Moreover, these polymorphisms are best suited for allele specific quantitative approaches. Together, DIP-based chimerism analysis accounts for an unambiguous donor/recipient differentiation and highly quantitative chimerims monitoring.

Mentype® **DIPscreen** is a multiplex-PCR application developed to identify DIP polymorphisms that individually occur in donor or recipient, and, constitute informative loci. Thereby, a single multiplex-PCR simultaneously screens 33 DIP-markers together with the gender specific locus Amelogenin. Identified informative DIP-loci can subsequently be addressed by Mentype® **DIPquant** specific real-time PCR assays to approach quantitative chimerism monitoring.
Thus, Mentype® **DIPscreen** initializes a highly quantitative chimerism analysis.

The 33 DIP loci addressed by Mentype® **DIPscreen** are distributed over 18 chromosomes, and are at least separated by 10 Mbp each (see Tab. 1). The detection limit of Mentype® **DIPscreen** is about **200 pg genomic DNA**. The optimal range under standard conditions is **1.0 -2.0 ng DNA.** For fast and sensitive fragment length analysis primers are fluorescence-labelled with **6-FAM**, **BTG**, or **BTY**.

The test kit was validated and evaluated using the GeneAmp® 9700 Silver, Eppendorf Mastercycler ep-S, Biometra T1, ABI PRISM® 3130 Genetic Analyzer running with 36 cm capillary array and POP4® polymer. Development, manufacture and distribution of Biotype® products are certified according to DIN EN ISO 9001:2008.

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## 1. Description of the Mentype® DIPscreen

### Table 1. Locus-specific information of Mentype® DIPscreen

| DIP Locus | Chromosomal position | Motive (-DIP / +DIP) |
|---|---|---|
| **FAM Panel** | | |
| AM X | Xp22.1-22.3 | |
| AM Y | Yp11.2 | |
| HLD106 | 16q13 | -/AATGCGT |
| HLD70 | 6q16.1 | -/AGCA |
| HLD84 | 8q24.12 | -/CTTTC |
| HLD103 | 12q23.1 | -/GCTTATAA |
| HLD104 | 13q32.1 | -/ACTC |
| HLD116 | 18p11.22 | -/AGGTGTCGAACAACATGATAC |
| HLD112 | 17p12 | -/TTGTA |
| HLD307 | Xp11.23 | -/TCAACCAA |
| HLD310 | 2p22.3 | -/GTCTGGTT |
| HLD110 | 16q22.1 | -/TCCCTG |
| HLD133 | 3p22.1 | -/CAACCTGGATT |
| HLD79 | 7q31.2 | -/AATCT |
| HLD105 | 14q24.3 | -/ATAGACAA |
| HLD140 | 3q23 | -/GGTAGTATGGGCCT |
| HLD163 | 12q24.31 | -/AACTACGGCACGCCC |
| **BTG Panel** | | |
| HLD91 | 11q14.1 | -/GATA |
| HLD23 | 18p11.32 | -/CTTTAA |
| HLD88 | 9q22.33 | -/CCACAAAGA |
| HLD101 | 15q26.1 | -/GTAG |
| HLD67 | 5q33.3 | -/CTACTGAC |
| HLD301 | 17q21.32 | -/CAGGGGCTC |
| HLD53 | 3q22.1 | -/ATGT |
| HLD97 | 13q13.1 | -/AGAGAAAGCTGAAG |
| HLD152 | 16p13.2 | -/TGGTCAAAGGCA |
| HLD128 | 1q31.3 | -/ATTAAATA |
| HLD134 | 5q11.2 | -/ATGATGGTTCTTCAGA |
| HLD305 | 20q11.22 | -/CAAGGTCCCACCACACTCGCGTGGGA |
| **BTY Panel** | | |
| HLD48 | 2q11.2 | -/GACTT |
| HLD114 | 17p13.2 | -/TCCTATTCTACTCTGAAT |
| HLD304 | 9q34.3 | -/GAGCTGCTCAAGAGAGAGG |
| HLD131 | 7q36.2 | -/TTGGGCTTATT |
| HLD38 | 1q32.2 | -/TAGTT |
| HLD82 | 7q21.3 | -ACCTCCTACTCCTTGGTCTATTCCTGGTCACATGTACT |

Abbreviations: HLD = Human Locus DIP, -DIP = Deletion, +DIP = Insertion

Table 1 shows the chromosomal position, motif and respective reference allele of DIP-loci addressed by Mentype® **DIPscreen**.

## Kit Content

Mentype® **DIPscreen** (100 Reactions)

| | |
|---|---|
| Nuclease-free water | 3.0 ml |
| Reaction mix **A** | 500 µl |
| Primer mix | 500 µl |
| Multi Taq2 DNA polymerase | 60 µl |
| Control DNA XY13 (2ng/µl) | 10 µl |
| DNA Size Standard 550 (BTO) | 50 µl |
| Allelic ladder | 25 µl |

## Ordering information

| | | | |
|---|---|---|---|
| Mentype® **DIPscreen** | 25 reactions | Cat. No. | 45-45410-0025 |
| Mentype® **DIPscreen** | 100 reactions | Cat. No. | 45-45410-0100 |
| Mentype® **DIPscreen** | 400 reactions | Cat. No. | 45-45410-0400 |

## Storage

Store all components at -20 °C and avoid repeated thawing and freezing. Primer mix and allelic ladder must be stored protected from light. The DNA samples and post-PCR reagents (allelic ladder and DNA size standard) should be stored separately from the PCR reagents. The expiry date is indicated on the kit cover.

## Additionally required reagents

Additional reagents are required in order to use the Biotype® PCR Amplification Kit:

| **Reagent** | **Supplier** | **Order Number** |
|---|---|---|
| Hi-Di™ Formamide, 25 ml | Applied Biosystems | 4311320 |
| Matrix Standards BT5 single-capillary instruments (5x25 µl) | Biotype Diagnostic GmbH | 00-10411-0025 |
| Matrix Standards BT5 multi-capillary instruments (25 µl) | Biotype Diagnostic GmbH | 00-10421-0025 |
| Matrix Standards BT5 multi-capillary instruments (50 µl) | Biotype Diagnostic GmbH | 00-10421-0050 |

## Warning and safety instructions

The PCR Amplification Kit contains the following potentially hazardous chemicals:

| Kit component | Chemical | Hazards |
|---|---|---|
| Reaction mix | Sodium azide $NaN_3$ | toxic if swallowed, develops toxic gases when it gets in contact with acids |

Observe the Material Safety Data Sheets (MSDS) for all Biotype® products, which are available on request. Please contact the respective manufacturers for copies of the MSDS for any additionally needed reagents.

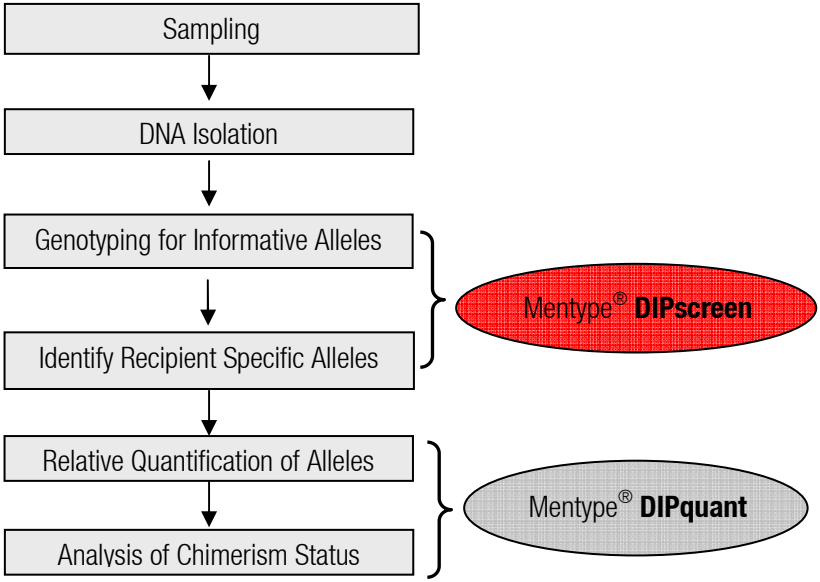
## Quality assurance

All kit components undergo an intensive quality assurance process at Biotype Diagnostic GmbH. The quality of the test kits is permanently monitored to ensure unrestricted usability. Please contact us if you have any questions regarding quality assurance.

## Trademarks and patents

Mentype® is a registered trademark of Biotype Diagnostic GmbH.
ABI PRISM®, GeneMapper® GeneAmp® and Applied Biosystems® are registered trademarks of Applied Biosystems LLC.
Under the law of Europe POP4® is registered trademark of Applied Biosystems LLC.
The PCR is covered by patents. Patentees are Hoffmann-La Roche Inc. and F. Hoffmann-La Roche (Roche).

**Outline of working steps performed with Mentype® DIP-products**




From sample to analysis – Monitoring chimerism with the Mentype® **DIPscreen** and the Mentype® **DIPquant** assay

## Protocols for PCR amplification, electrophoresis and analysis

### 2. PCR amplification

### 2.1 Master mix preparation

The table below shows the volumes of all PCR reagents per 25 µl reaction volume, including a sample volume of 1.0 µl (template DNA). The number of reactions to be set up shall be determined taking into account positive and negative control reactions. Add one or two reactions to this number to compensate the pipetting error.

| Component | Volume |
|---|---|
| Nuclease-free water | 13.4 µl |
| Reaction mix **A*** | 5.0 µl |
| Primer mix | 5.0 µl |
| Multi Taq2 DNA polymerase (hot start, 2.5 U/µl) | 0.6 µl |
| Volume of master mix | 24.0 µl |

* contains $Mg^{2+}$, dNTPs, BSA

All components should be mixed (vortex) and centrifuged for about 10 s before preparing the master mix. The DNA volume applied to the assay depends on its concentration. For reference samples 1 µl is mostly sufficient. For critical patient samples the amount of template can be increased appropriately. Fill up the final reaction volume to 25 µl with nuclease-free water.

Generally, DNA templates shall be stored in nuclease-free water or in diluted TE buffer (10 mM Tris HCl, pH 8.0 and 1 mM EDTA), e.g. 0.1x TE buffer.

The primer mixes are adjusted for balanced peak heights at **28 PCR cycles** and **1 ng Control DNA XY13** in a reaction volume of 25 µl. If more DNA template is applied, higher peaks can be expected for small PCR fragments and relatively low peaks for large fragments. Reduce the amount of DNA template to correct this imbalance.

#### Positive control

For the positive amplification control, dilute the Control DNA XY13 to 1 ng/µl. Instead of the template DNA, pipette the diluted Control DNA into a reaction tube containing the PCR master mix.

#### Negative control

For the negative amplification control, pipette nuclease-free water instead of template DNA into a reaction tube that contains the PCR master mix.

#### Template DNA

Sometimes, measured DNA concentration varies depending on the quantification method used. It might thus be necessary to adjust the optimal DNA amount.

## 2.2 PCR amplification parameter

Perform a “hot start” PCR in order to activate the Multi Taq2 DNA Polymerase and to prevent formation of non-specific amplification products.

The number of cycles depends on the amount of DNA applied. 28 PCR cycles are recommended for all samples.

### Standard method

Recommended for all DNA samples

| **Temperature** | **Time** | |
|---|---|---|
| 94°C | 4 min (hot start for activation of the Multi Taq2 DNA polymerase) | |
| 94°C | 30 s | |
| **60°C** | **120 s** | **28 cycles** |
| 72°C | 75 s | |
| 68°C | 60 min | |
| 10°C | ∞ | hold |

Note: To provide an optimal kit balance the ramping rate of the thermal cycler should be adjusted to 4-5 °C/s.

Very small amounts of DNA may result in statistical dropouts and imbalances of the peaks. Increasing numbers of PCR cycles raise the risk of cross contamination caused by minimal amounts of impurities. Furthermore, unspecific amplification products could appear.

## 3. Electrophoresis using the ABI PRISM® 310 Genetic Analyzer

For general instructions on instrument setup, matrix generation and application of the GeneScan® or GeneMapper® ID software, refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual*. Electrophoresis using the GeneScan® software is described below.

The virtual **filter set G5** shall be used for combined application of the five fluorescent labels **6-FAM**, **BTG**, **BTY, BTR, and BTO** (the matrix standard will be called **BT5** hereinafter).

| **Material** | |
|---|---|
| Capillary | 47 cm / 50 µm (green) |
| Polymer | POP4® for 310 Genetic Analyzer |
| Buffer | 10x Genetic Analyzer Buffer with EDTA |

### 3.1 Matrix generation

Prior to conducting DNA fragment size analysis with the **Filter Set G5**, a matrix with the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, and **BTO** must be generated.

| **Color** | **Matrix standard** |
|---|---|
| Blue (B) | 6-FAM |
| Green (G) | BTG |
| Yellow (Y) | BTY |
| Red (R) | BTR |
| Orange (O) | BTO |

Five electrophoresis runs shall be conducted, one for each fluorescent label, **6-FAM**, **BTG**, **BTY**, **BTR**, and **BTO**. Use the same conditions as for samples and allelic ladders of the Biotype® test kit to generate suitable matrix files.

| **Matrix sample** | **Component** | **Volume** |
|---|---|---|
| Matrix sample 1 | Hi-Di™ Formamide | 12.0 µl |
| | Matrix standard **6-FAM** | 1.0 µl |
| Matrix sample 2 | Hi-Di™ Formamide | 12.0 µl |
| | Matrix standard **BTG** | 1.0 µl |
| Matrix sample 3 | Hi-Di™ Formamide | 12.0 µl |
| | Matrix standard **BTY** | 1.0 µl |
| Matrix sample 4 | Hi-Di™ Formamide | 12.0 µl |
| | Matrix standard **BTR** | 1.0 µl |
| Matrix sample 5 | Hi-Di™ Formamide | 12.0 µl |
| | Matrix standard **BTO** | 1.0 µl |

- Denaturation for 3 min at 95°C
- Cool down to 4°C and place samples on the autosampler tray

- Create a **Sample Sheet** choose **5 Dyes** and enter a sample designation

## Injection list for matrix generation

| Parameter | Set up |
|---|---|
| Module File | GS STR POP4 (1 ml) **G5** |
| Matrix File | **NONE** |
| Size Standard* | **NONE** |
| Injection [s] | 5 |
| Injection [kV] | 15.0 |
| Run [kV] | 15.0 |
| Run [°C] | 60 |
| Run Time [min] | 24 |

* Prepare matrix standards always **without DNA Size Standard (BTO)**

## Analysis of the matrix samples

- Run the GeneScan® software
- **File** → **New** → **Project** (open folder of current run) → **Add Sample Files**
- Select a matrix sample in the **Sample File** column
- **Sample** → **Raw Data**
- Check the matrix samples for a flat baseline. As shown in the figure below there should be at least five peaks with peak heights about 1000-4000 RFU (Y-axis) for each matrix sample (optimal range: 2000-4000 RFU)

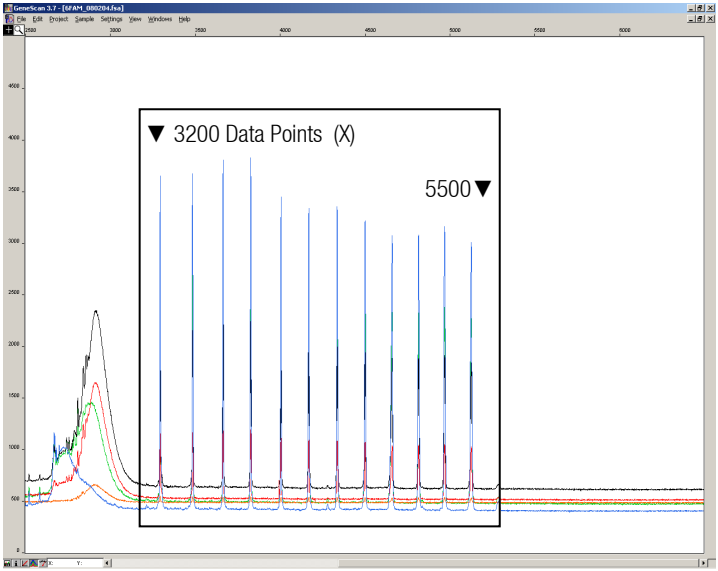



**Fig. 1** Electropherogram with raw data of the matrix standard 6-FAM

- Select an analysis range with flat baseline and re-inject the matrix sample if necessary
- Note down start and end value (data points) of the analysis range, e.g. start value 3200, end value 5500
- Calculate the difference, e.g. 5500-3200 = 2300 data points

## Generation of a new matrix

- **File** → **New** → **Matrix**

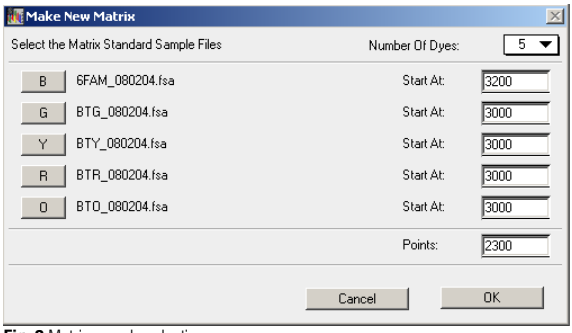



**Fig. 2** Matrix sample selection

- Import matrix samples for all dyes (B, G, Y, R, O)
- Enter a **Start At** value, e.g. 3200
- Enter the calculated difference under **Points**, e.g. 2300
- Click on **OK** to calculate the new matrix

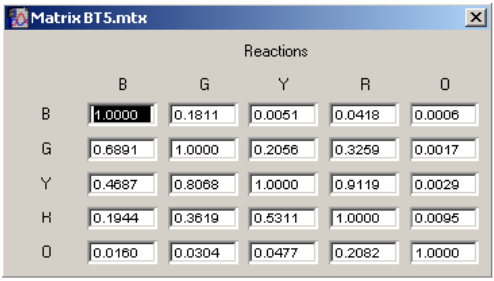



**Fig. 3** New matrix BT5

- Save the matrix in the matrix folder: **File** → **Save as**, e.g. Matrix BT5

## Matrix check

Check the new matrix with current samples.
- **File** → **New** → **Project** (open folder of the respective run) → **Add Sample Files**
- Select sample(s) in the **Sample File** column
- **Sample** → **Install New Matrix** (open matrix folder and select new matrix)
- Re-analyse your samples

There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix.

## 3.2 Sample preparation

| Component | Volume |
|---|---|
| Hi-Di™ Formamide | 12.0 µl |
| DNA Size Standard 550 (BTO) | 0.5 µl |
| Prepare 12 µl of the mix (formamide + DNA size standard) for all samples | |
| Add 1 µl PCR product (diluted if necessary) or allelic ladder | |

- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

### Signal intensities

### Options to increase the signal intensity:

- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

## 3.3 Setting up the Data Collection Software

- Create a **Sample Sheet** and enter a sample designation

### Injection list

| Parameter | Set up |
|---|---|
| Module File | GS STR POP4 (1 ml) **G5** |
| Matrix File | e.g. Matrix BT5 |
| Size Standard | e.g. SST-BTO_60-450bp |
| Injection [s]* | 5 |
| Injection [kV] | 15.0 |
| Run [kV] | 15.0 |
| Run [°C] | 60 |
| Run Time [min]** | **26** |

***** Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low DNA content an injection time of up to 20 s may be necessary.

****** Depending on the analysis conditions, the run time for Mentype® **DIPscreen** was modified in order to be able to analyse fragments with lengths of up to **450 bp**.

### 3.4 Analysis parameter

The recommended analysis parameters are:

| Analysis Range | Full Range |
|---|---|
| Data Processing | Baseline: Checked<br>Multicomponent: Checked<br>Smooth Options: Light |
| Peak Detection | Peak Amplitude Thresholds<br>B:* Y:*<br>G:* R:*<br>O:*<br>Min. Peak Half Width: 2 pts<br>Polynominal Degree: 3<br>Peak Window Size: 15 pts |
| Size Call Range | Min: 60<br>Max: 550 |
| Size Calling Method | Local Southern Method |
| Split Peak Correction | None |

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneScan® or GeneMapper® ID software. Thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

## 4. Electrophoresis using the ABI PRISM® 3100-Avant/3100 Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, application of the ABI PRISM® 3100 Data Collection Software version 1.01 or 1.1 and the GeneScan® software, refer to the *ABI PRISM® 3100-Avant/3100 Genetic Analyzer User's Manual*. For systems with Data Collection Software 2.0 or 3.0 refer to chapter 5.

The system with 4 capillaries is named ABI 3100-Avant, and the system with 16 capillaries is named ABI 3100.

The virtual **filter set G5** shall be used for combined application of the five fluorescent labels **6-FAM**, **BTG**, **BTY, BTR, and BT0** (the matrix standard will be called **BT5** hereinafter).

**Material**

| | |
|---|---|
| Capillary | 36 cm Capillary Array for 3100-Avant/3100 |
| Polymer | POP-4® Polymer for 3100 |
| Buffer | 10x Genetic Analyzer Buffer with EDTA |

### 4.1 Spectral calibration / matrix generation

Proper spectral calibration is critical to evaluate multicolour systems with the ABI PRISM® 3100-Avant/3100 Genetic Analyzer and shall be done prior to conducting fragment length analysis with the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, and **BT0**. The calibration procedure creates a matrix which is used to correct the overlapping of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation of the spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Entering the plate composition
- Performing a spectral calibration run and checking the matrix

#### Setting up the spectral calibration standards

Example for 4 capillaries / ABI 3100-Avant

| **Component** | **Volume** |
|---|---|
| Hi-Di™ Formamide | 60.0 µl |
| Matrix standard **BT5** | 5.0 µl |

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-D1**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

Example for 16 capillaries / ABI 3100

| **Component** | **Volume** |
|---|---|
| Hi-Di™ Formamide | 204.0 µl |
| Matrix standard **BT5** | 17.0 µl |

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-H1** and **A2-H2**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples on the autosampler tray

### Performing a spectral calibration run

First of all, the parameter file for **DyeSetG5** must be modified once to achieve successful calibration with the Data Collection software version 1.0.1 or 1.1.

### Spectral parameter

To change settings in the parameter file go to the following path:
D:\AppliedBio\Support Files\Data Collection Support Files\CalibrationData\Spectral Calibration\ParamFiles

- Select **MtxStd{Genescan_SetG5}** to open the PAR-file
- Change **Condition Bounds Range** to [1.0; 20.0]
- Select **File** → **Save As** to save the parameter file under a new name, e.g. MtxStd{Genescan_SetG5_BT5}.par

Always use this parameter file for spectral calibration runs using Biotype® matrix standards **BT5**.

### Plate Editor for spectral calibration (I)

- Place the 96-well plate on the autosampler tray
- Run the ABI PRISM® 3100 Data Collection software
- In **Plate View** click **New** to open the **Plate Editor** dialog box
- Enter a name of the plate
- Select **Spectral Calibration**
- Select **96-Well** as plate type and click on **Finish**

### Plate editor for spectral calibration (II)

| **Parameter** | **Set up** |
|---|---|
| Sample Name | Type name for the matrix samples |
| Dye Set | G5 |
| Spectral Run Module | *Default* (e.g. Spect36_POP4®) |
| Spectral Parameters | MtxStd{GeneScan_SetG5_BT5}.par (parameters created before) |

- Click into the column header to select the entire column, select **Edit** → **Fill Down** to apply the information of the selected samples and confirm with **OK**
- Link your reaction plate on the autosampler tray with the created plate ID and start run
- On completion of the run check in the **Spectral Calibration Result** dialog box if all capillaries have successfully passed calibration (label **A**). If individual capillaries are labelled **X**, refer to *ABI PRISM® Genetic Analyzer User's Manual*.
- Click on **OK** to confirm completion of the run

### Matrix check

- Select **Tools → Display Spectral Calibration → Dye Set → G5** to review the spectral calibration profile for each capillary
- The quality value (**Q value**) must be greater than 0.95 and the condition number (**C value**) must be between 1 and 20. Both values must be within the previously determined range
- Check the matrix samples for a flat baseline. There should be five peaks with peak heights of about 1000-5000 RFU (Y-axis) in each matrix sample (optimal range: 2000-4000 RFU)
- If all capillaries have passed the calibration, the last calibration file for **Dye Set G5** must be activated manually under **Tools → Set Active Spectral Calibration**. Rename the calibration file under **Set Matrix Name** (e.g. BT5_Date of calibration)
- If calibration was not successful, try to re-inject the samples with higher injection voltage or injection time. The editing of the Spectral Run Module will be necessary. You can re-inject the same samples up to three times. Otherwise use more matrix standard for spectral calibration
- Check the new matrix with your current samples. There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix

## 4.2 Sample preparation

| **Component** | **Volume** |
|---|---|
| Hi-Di™ Formamide | 12.0 µl |
| DNA Size Standard 550 (BTO) | 0.5 µl |
| Prepare 12 µl of the mix (formamide + DNA size standard) for all samples | |
| Add 1 µl PCR product (diluted if necessary) or allelic ladder | |

- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place the samples on the autosampler tray

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted on the plate of multi-capillary analyzers. If fewer samples are analysed, the empty positions must be filled with 12 µl Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analyzers, several ladders should be run.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Pay attention to keeping ambient conditions as recommended by the instrument manufacturer. Optimal will be a stable room temperature > 22 °C.

### Signal intensities

**Options to increase the signal intensity:**
- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

## 4.3 Setting up the Data Collection Software

Edit the default run module in **Dye Set G5** once for the first run.
- Select **Module Editor** to open the dialog box
- Select the appropriate **Run Module** as template from the **GeneScan** table
- Modify the **Injection Voltage** to 3 kV and the **Injection Time** to 10 s

### Run Module 3kV_10s_450bp

| **Parameter** | **Set up** |
|---|---|
| Run Temperature [°C] | *Default* |
| Cap Fill Volume | *Default* |
| Maximum Current [A] | *Default* |
| Current Tolerance [A] | *Default* |
| Run Current [A] | *Default* |
| Voltage Tolerance [kV] | *Default* |
| Pre Run Voltage [kV] | *Default* |
| Pre Run Time [s] | *Default* |
| Injection Voltage [kV] | **3.0** |
| Injection Time [s]* | **10** |
| Run Voltage [kV] | *Default* |
| Number of Steps | *Default* |
| Voltage Step Interval | *Default* |
| Data Delay Time [s] | *Default* |
| Run Time [min]** | **25** |

***** Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low DNA content an injection time of up to 20 s may be necessary.

****** Depending on the analysis conditions, the Run Time for Mentype® **DIPscreen** was modified in order to be able to analyse fragments with lengths of up to **450 bp**.

- Click on **Save As**, enter the name of the new module (e.g. 3kV_10s_450bp) and confirm with **OK**
- Click on **Close** to exit the **Run Module Editor**

### Starting the run

- Place the prepared 96-well plate on the autosampler tray
- Run the ABI PRISM® 3100 Data Collection software
- In **Plate View** click on **New** to open the **Plate Editor** dialog box
- Enter a name of the plate
- Select **GeneScan**

- Select **96-Well** as plate type and click on **Finish**

### Plate Editor

| **Parameter** | **Set up** |
|---|---|
| Sample Name | enter a name |
| Dyes | O |
| Colour Info | Ladder or sample |
| Project Name | e.g. 3100_Project1 |
| Dye Set | G5 |
| Run Module* | 3kV_10s_450bp |
| Analysis Module 1 | DefaultAnalysis.gsp |

* parameter see above

- Complete the table in the **Plate Editor** and click on **OK**
- Click into the column header to select the entire column and select **Edit → Fill Down** to apply the information of the selected samples
- Link your reaction plate on the autosampler tray with the created plate ID and start the run
- On completion of the run, view data as **Color Data** in **Array View** of the 3100 Data Collection software or as **Analyzed Sample Files** under D:/AppliedBio/3100/DataExtractor/ExtractRuns

## 4.4 Analysis parameter / analysis method

The recommended analysis parameters are:

| Analysis Range | Full Range |
|---|---|
| Data Processing | Baseline: Checked<br>Multicomponent: Checked<br>Smooth Options: Light |
| Peak Detection | Peak Amplitude Thresholds<br>B:* Y:*<br>G:* R:*<br>O:*<br>Min. Peak Half Width: 2 pts<br>Polynominal Degree: 3<br>Peak Window Size: 15 pts |
| Size Call Range | Min: 60<br>Max: 550 |
| Size Calling Method | Local Southern Method |
| Split Peak Correction | None |

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneScan® or GeneMapper® ID software. Thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

## 5. Electrophoresis using the ABI PRISM® 3130/3130xl Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, or application of the ABI PRISM® Data Collection software version 3.0 and the GeneMapper® ID/ID-X software, refer to the *ABI PRISM® 3130/3130xl Genetic Analyzers Getting Started Guide*.

The system with 4 capillaries is named ABI 3130, and the system with 16 capillaries is named ABI 3130xl.

The virtual **filter set Any5Dye** shall be used for the combined application of the five fluorescent labels **6-FAM**, **BTG**, **BTY, BTR, and BTO** (the matrix standard will be called **BT5** hereinafter).

**Material**

| | |
|---|---|
| Capillary | 36 cm Capillary Array for 3130/3130xl |
| Polymer | POP4® Polymer for 3130 |
| Buffer | 10x Genetic Analyzer Buffer with EDTA |

### 5.1 Spectral calibration / matrix generation

Prior to conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, and **BTO** for each analyzer. The calibration procedure creates a matrix which is used to correct the overlapping of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation the spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Creating the instrument protocol for spectral calibration (Protocol Manager)
- Defining the plate composition in the plate editor (Plate Manager)
- Performing a spectral calibration run and checking the matrix

## Setting up the spectral calibration standards

Example for 4 capillaries/ABI 3130

| **Component** | **Volume** |
|---|---|
| Hi-Di™ Formamide | 60.0 µl |
| Matrix standard **BT5** | 5.0 µl |

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-D1**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples in the autosampler tray

Example for 16 capillaries/ABI 3130xl

| **Component** | **Volume** |
|---|---|
| Hi-Di™ Formamide | 204.0 µl |
| Matrix standard **BT5** | 17.0 µl |

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-H1** and **A2-H2**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples in the autosampler tray

## Performing a spectral calibration run

- Place the 96-well plate on the autosampler tray
- In the **Protocol Manager** of the Data Collection software click on **New** in **Instrument Protocol** to open the **Protocol Editor** dialog box

## Instrument Protocol for spectral calibration

| **Protocol Editor** | **Set up** |
|---|---|
| Name | *User* (e.g. Spectral36_POP4_BT5) |
| Type | SPECTRAL |
| Dye Set | **Any5Dye** |
| Polymer* | *User* (e.g. POP4) |
| Array Length* | *User* (e.g. 36cm) |
| Chemistry | Matrix Standard |
| Run Module* | *Default* (e.g. Spect36_POP4_1) |

* Depends on the type of polymer and length of capillary used

- Click on **OK** to leave the **Protocol Editor** dialog box
- In the **Plate Manager** of the Data Collection software, click on **New** to open the **New Plate Dialog** box

## Plate Editor for spectral calibration (I)

| **New Plate Dialog** | **Set up** |
|---|---|
| Name | e.g. Spectral_BT5_date |
| Application | Spectral Calibration |
| Plate Type | 96-Well |
| Owner Name / Operator Name | … |

- Click on **OK**. A new table in the **Plate Editor** will open automatically

## Plate Editor for spectral calibration (II)

| **Parameter** | **Set up** |
|---|---|
| Sample Name | Enter name for the matrix samples |
| Priority | e.g. 100 |
| Instrument Protocol 1 | Spectral36_POP4_BT5 (setting described before) |

- Click into the column header to select the entire column, select **Edit** → **Fill Down** to apply the information to all selected samples, and click on **OK**
- In the **Run Scheduler** click on **Find All**, select **Link** to link the reaction plate on the autosampler up with the newly created plate record (position A or B) and start the run

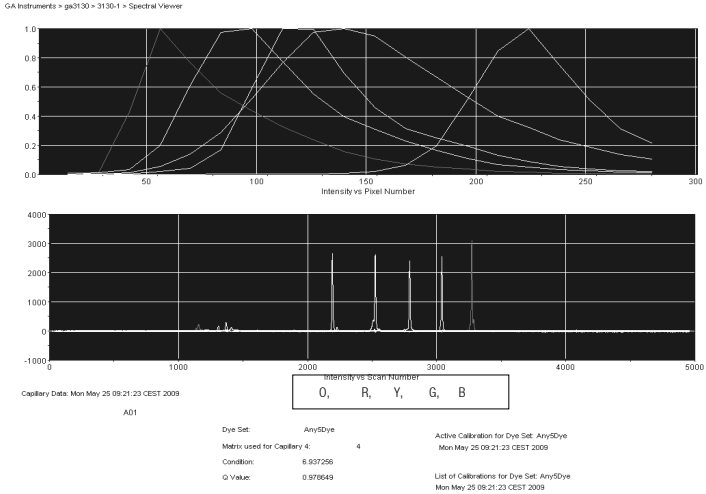

GA Instruments > ga3130 > 3130-1 > Spectral Viewer
Intensity vs Pixel Number
Intensity vs Scan Number
O, R, Y, G, B
Capillary Data: Mon May 25 09:21:23 CEST 2009
A01
Dye Set: Any5Dye
Matrix used for Capillary 4: 4
Condition: 6.937256
Q Value: 0.978649
Active Calibration for Dye Set: Any5Dye
Mon May 25 09:21:23 CEST 2009
List of Calibrations for Dye Set: Any5Dye
Mon May 25 09:21:23 CEST 2009

**Fig. 4** Electropherogram of spectral calibration with matrix standard BT5 on an ABI 3130

## Matrix check

- The quality value (**Q value**) of each capillary must be greater than 0.95 and the condition number range (**C value**) must be between 1 and 20
- Check the matrix samples for a flat baseline. As shown in the figure above, there should be five peaks with peak heights of about 1000-5000 RFU (Y-axis) in each matrix sample (optimal range: 2000-4000 RFU)
- If all capillaries have passed the test, the last calibration file for the Dye Set **Any5Dye** is activated automatically in the **Spectral Viewer**. **Rename** the calibration file (e.g. BT5_Date of calibration) using the respective button
- If calibration was not successful, try to re-inject the samples with higher injection voltage or injection time. Editing of the Spectral Run Module will be necessary. You can re-inject the same samples up to three times. Otherwise use more matrix standard for spectral calibration
- Check the new matrix with your current samples. There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix

## Sample preparation

| Component | Volume |
|---|---|
| Hi-Di™ Formamide | 12.0 µl |
| DNA Size Standard 550 (BTO) | 0.5 µl |
| Prepare 12 µl of the mix (formamide + DNA size standard) for all samples | |
| Add 1 µl PCR product (diluted if necessary) or allelic ladder | |

- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place the samples on the tray

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted on the plate of multi-capillary analyzers. If fewer samples are analysed, the empty positions must be filled with 12 µl Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analysers, several ladders should be run.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Pay attention to keeping ambient conditions as recommended by the instrument manufacturer. Optimal will be a stable room temperature > 22 °C.

## Signal intensities

### Options to increase the signal intensity:

- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

## 5.2 Setting up the Data Collection Software

Edit the run module as follows for the first run:

- In the **Module Manager** of the Data Collection Software click on **New** to open the **Run Module Editor** dialog box

### Run Module 3kV_10s_450bp

| **Parameter** | **Set up** |
|---|---|
| Oven Temperature [°C] | *Default* |
| Poly Fill Volume | *Default* |
| Current Stability [µA] | *Default* |
| PreRun Voltage [kV] | *Default* |
| PreRun Time [s] | *Default* |
| Injection Voltage [kV] | **3.0** |
| Injection Time [s]* | **10** |
| Voltage Number of Steps | *Default* |
| Voltage Step Interval | *Default* |
| Data Delay Time [s] | *Default* |
| Run Voltage [kV] | *Default* |
| Run Time [s]** | **1500** |

***** Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If references samples with very high signal intensities are recorded, a shorter injection time may be selected in orer to avoid pull-up peaks. For samples with low DNA content an injection time of up to 20 s my be necessary.

****** Depending on the analysis conditions the run time for Mentype® **DIPscreen** was modified in order to be able to analyse fragments with lengths of up to **450 bp**.

- Click on **Save As**, enter the name of the new module (e.g. 3kV_10s_450bp) and confirm with **OK**
- Click on **Close** to exit the **Run Module Editor**

### Starting the run

- Place the prepared 96-well plate on the autosampler tray
- In the **Protocol Manager** of the Data Collection software, click on **New** in the **Instrument Protocol** window to open the **Protocol Editor** dialog box

### Instrument Protocol

| **Protocol Editor** | **Set up** |
|---|---|
| Name | enter a name |
| Type | REGULAR |
| Run Module* | 3kV_10s_450bp |
| Dye Set | **Any5Dye** |

* parameter see above

- Click on **OK** to exit the **Protocol Editor**

Prior to each run, it is necessary to create a plate definition as follows:

- In the **Plate Manager** of the Data Collection software click on **New** to open the **New Plate Dialog** box

### Plate Editor (I)

| **New Plate Dialog** | **Set up** |
|---|---|
| Name | e.g. Plate_BT5_Date |
| Application | Select GeneMapper Application |
| Plate Type | 96-Well |
| Owner Name / Operator Name | … |

- Click on **OK**. A new table in the **Plate Editor** will open automatically

### Plate Editor (II)

| **Parameter** | **Set up** |
|---|---|
| Sample Name | Enter name for the samples |
| Priority | e.g. 100 (Default) |
| Sample Type | Sample or allelic ladder |
| Size Standard | e.g. SST-BTO_60-450bp |
| Panel | e.g. DIPscreen_Panels_v0 |
| Analysis Method | e.g. Analysis_DIPscreen_3130_200rfu |
| Snp Set | - |
| User-defined 1-3 | - |
| Results Group 1 | (select results group) |
| Instrument Protocol 1 | Run36_POP4_BT5_25min (setting described before) |

- Click into the column header to select the entire column, select **Edit → Fill Down** to apply the information to all selected samples and click on **OK**
- In the **Run Scheduler,** click on **Find All**, select **Link** to link the reaction plate on the autosampler up with the newly created plate record (position A or B) and start the run
- During the run, view **Error Status** in the **Event Log** or examine the quality of the raw data for each capillary in the **Capillaries Viewer** or the **Cap/Array Viewer**
- View data as overview in **Run History** or **Cap/Array Viewer** of the Data Collection software. Run data are saved in the **Run Folder** of the previously chosen **Result Group**

## 5.3 Analysis parameter / analysis method

The recommended analysis parameters are:

| Peak Detection Algorithm | Advanced |
|---|---|
| Ranges | Analysis: Full Range<br>Sizing: All Sizes |
| Smoothing and Baselining | Smoothing: Light<br>Baseline Window: 51 pts |
| Size Calling Method | Local Southern Method |
| Peak Detection | Peak Amplitude Thresholds<br>B:* Y:*<br>G:* R:*<br>O:*<br>Min. Peak Half Width: 2 pts<br>Polynominal Degree: 3<br>Peak Window Size: 15 pts<br>Slope Thresholds: 0.0 |

Recommend settings in the worksheet **Allele** are:

| | |
|---|---|
| Amelogenin Cutoff** | 0.1 |

Recommend settings in the worksheet **Peak Quality** are:

| | |
|---|---|
| Heterozygote balance | Min peak height ratio: 0.1 |
| Allele number | Max expected alleles: 2 |

* The peak amplitude threshold (Cutoff value) corresponds to the minimum peak height that will be detected from the GeneMapper® ID/ID-X software. The thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times higher then the background noise of the baseline.

** All DIPs will be examined by GeneMapper® ID/ID-X software like Amelogenin.

## 6. Electrophoresis using the ABI PRISM® 3500/3500xL Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, or application of the Applied Biosystems 3500 Series Data Collection Software version 1.0 and the GeneMapper® ID-X software version 1.2, refer to the *Applied Biosystems 3500/3500xL Genetic* Analyzers User Guide.

The system with 8 capillaries is named AB 3500 and the system with 24 capillaries is named AB 3500xL.

The virtual **filter set Any5Dye** shall be used for the combined application of five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, and **BTO** (the matrix standard will be called **BT5** hereinafter).

**Material**

| | |
|---|---|
| Capillary | 36 cm Capillary Array for 3500/3500xL |
| Polymer | POP-4® Polymer for 3500/3500xL |
| Buffer | 10x Genetic Analyzer Buffer with EDTA for 3500/3500xL |

### 6.1 Spectral calibration / matrix generation

Prior to conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, and **BTO** for each analyzer. The calibration procedure creates a matrix that is used to correct the overlap of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation of spectral calibration standards
- Loading the standards to the multi-well reaction plate (one sample per capillary)
- Preparation of instrument and creating a Dye Set BT5
- Performing a spectral calibration run and checking the matrix

### Setting up the spectral calibration standards

Example for 8 capillaries/ABI 3500

| Component | Volume |
|---|---|
| Hi-Di™ Formamide | 108.0 µl |
| Matrix standard **BT5** | 9.0 µl |

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-H1**
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples in the autosaple tray

Example for 24 capillaries/ABI 3500xL

| Component | Volume |
|---|---|
| Hi-Di™ Formamide | 300.0 µl |
| Matrix standard **BT5** | 25.0 µl |

- Load 12 µl of the mix to a 96-well reaction plate, e.g. position **A1-H1**, **A2-H2 and A3-H3***
- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place samples in the autosample tray

* When using a 384-well plate, load 10 µl of the mixtures to columns 1, 3, and 5 in rows A, C, E, G, I, K, M, and O.

### Performing a spectral calibration run

- Place the multi-well plate on the autosampler tray
- Now prepare the instrument and specific spectral calibration run settings

### Preparation of the instrument

Before starting the spectral calibration process ensure that the spatial calibration has been performed. This process is necessary if a new capillary array was installed before and is described in detail in the *Applied Biosystems 3500/3500xL Genetic Analyzers User Guide*.

### Preparation of dye set BT5

Prior to the spectral calibration, a dye set for the Matrix Standard BT5 needs to be setup.

1. To create a new dye set, go to **Library** and select **Analyze**, followed by **Dye Sets** and click **Create**.
2. Enter a **Dye Set Name**, e.g. BT5.
3. Select **Matrix Standard** as a chemistry and **AnyDye Template** as a Dye Set Template.
4. Disable **Purple** in the field **Arrange Dyes**. Ensure that all other colors are enabled.
5. Under **Calibration Peak Order** the colors need to be arranged as Follows: **5 – blue**, **4 – green**, **3 – yellow**, **2 – red**, and **1 – orange**.
6. Do not alter the **Parameter** settings.
7. Click **Save** to confirm the changes.

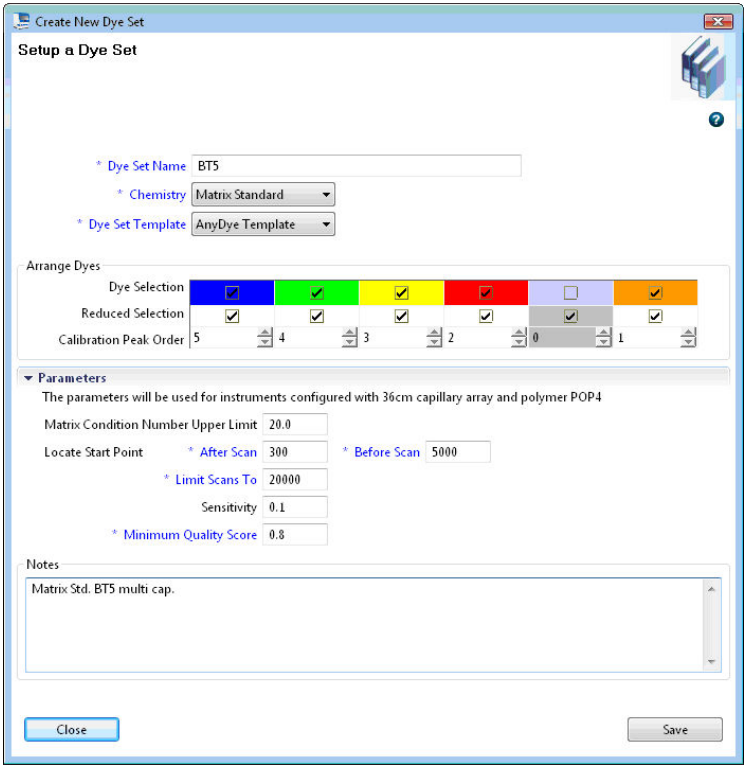



**Fig. 5** Setup for dye set BT5

- In the **Protocol Manager** of the Data Collection software click on **New** in **Instrument Protocol** to open the **Protocol Editor** dialog box

## Performing a spectral calibration run

Once the multiwell plate containing the spectral calibration mixture is placed in the autosampler tray the spectral calibration process can be started.

1. To access the Spectral Calibration screen, select **Maintenance** on the Dashboard of the 3500 Series Data Collection software.
2. The number of wells in the spectral calibration plate and their location in the instrument must be specified.
3. Select **Matrix Standard** as a chemistry standard and **BT5** for dye set.
4. (Optional) Enable **Allow Borrowing**.
5. Click **Start Run**.

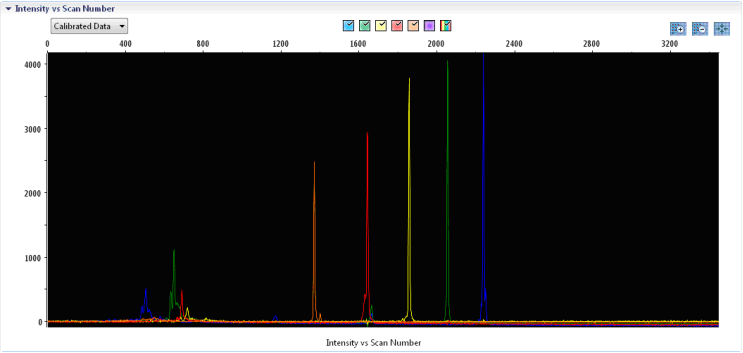



**Fig. 6** Electropherogram of spectral calibration with matrix standard BT5 on an ABI 3500

### Matrix check

- The quality value (**Q value**) of each capillary must be greater than 0.8 and the condition number range (**C value**) must be between 1 and 20
- Check the matrix samples for a flat baseline. As shown in the figure above, there should be five peaks with peak heights of about 1000-5000 RFU (Y-axis) in each matrix sample (optimal range: 2000-4000 RFU)
- A successful calibration will be displayed in green in **Overall** and for each capillary
- If all capillaries have passed the test, **Accept Results**
- If calibration failed, **Reject Results** and refer to **spectral calibration troubleshooting** of Applied Biosystems 3500/3500xL Genetic Analyzer User Guides

## 6.2 Sample preparation

| **Component** | **Volume** |
|---|---|
| Hi-Di™ Formamide | 12.0 µl |
| DNA Size Standard 550 (BTO) | 0.5 µl |
| prepare 12 µl of the mix (formamide + DNA size standard) for all samples | |
| add 1 µl PCR product (diluted if necessary) or allelic ladder | |

- Denaturation for 3 min at 95 °C
- Cool down to 4 °C and place the samples on the autosample tray

Since injections take place simultaneously on all capillaries, 8 or 24 samples must be pipetted on the plate of multi-capillary analysers. If fewer samples are analysed empty positions need to be filled with 12 µl Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analysers, several ladders should be run.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Pay attention to keeping ambient conditions as recommended by the instrument manufacturer. Optimal will be a stable room temperature > 22 °C.

### Signal intensities

**Options to increase the signal intensity:**

- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

## 6.3 Setting up a run

For the first run using the Mentype® **DIPscreen** Kit you will need to setup a number of protocols within the 3500 Series Data Collection Software.

### Create Instrument protocol

- Go to **Library** and select **Analyze** / **Instrument protocol** and click **Create**
- Change the parameters according the table below

### Instrument protocol for Mentype® DIPscreen®

| **Parameter** | **Set up** |
|---|---|
| Application Type | HID / Microsatellite |
| Capillary Length | *Default* |
| Polymer | *Default* |
| Dye Set | BT5 |
| Run Module | *Default* |
| Protocol Name | e.g. Mentype DIPscreen |
| Oven Temperature [°C] | *Default* |
| Run Voltage [kV] | *Default* |
| Injection Voltage [kV] | **3.0** |
| Run Time [s]** | **1500** |
| PreRun Time [s] | *Default* |
| Injection Time [s]* | **10** |
| Data Delay Time [s] | *Default* |
| Advanced Options | *Default* |

***** Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If reference samples with very high signal intensities are recorded, a shorter injection time may be selected in order to avoid pull-up peaks. For samples with low DNA content an injection time of up to 20 s may be necessary.

****** Depending on the analysis conditions, the run time for Mentype® **DIPscreen** was modified in order to analyse fragments with lengths of up to **450 bp**.

- Click on **Save** to confirm the settings

### Create Size Standard

- Go to **Library** and select **Analyze** / **Size Standards** and click **Create**
- Change the parameters according the table below

| Parameter | Set up |
|---|---|
| Size Standard | BTO_550 |
| Dye Color | Orange |

The DNA Size Standard 550 (BTO) should be used with the following lengths of fragments: **60**, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240, 250, 260, 280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550** bp.

- Click on **Save** to confirm the settings

### Create QC (Size Calling) Protocol

- Go to **Library** and select **Analyze** / **QC (Size Calling)** and click **Create**
- Change the parameters according the table below

| Parameter | Set up |
|---|---|
| Protocol Name | enter a name |
| Size Standard | BTO_550 (from above) |
| Sizecaller | Size Caller v.1.1.0 |

- Go to **Analysis Settings** / **Peak Amplitude Threshold** and **disable purple**. All other colours should be enabled.
- Keep all other settings as Default
- Click on **Save** to confirm the settings

### Create an Assay

- Go to **Library** and select **Manage** / **Assays** and click **Create**
- Change the parameters according the table below

| Parameter | Set up |
|---|---|
| Assay Name | e.g. Mentype DIPscreen |
| Color | Default |
| Application Type | HID |
| Instrument Protocol | e.g. Mentype DIPscreen |
| QC Protocols | e.g. BTO_550 |

- Click on **Save** to confirm the settings

**Starting the run**

- Place the prepared multi-well plate on the autosampler tray
- In the **Dashboard** of the Data Collection Software, click **Create New Plate**
- Go **to Define Plate Properties** and select **Plate Details**
- Change the parameters according the table below

**Plate Details**

| **Property** | **Set up** |
|---|---|
| Name | enter a name |
| Number of Wells | 96 or 384 |
| Plate Type* | HID |
| Capillary Lenght | **36cm** |
| Polymer | **POP4** |

- Click **Assign Plate Contents** to confirm the settings
- Define well position of each sample or ladder for data collection and processing by entering sample names
- Assign an **Assay** (required) a **File Name Conventions** and a **Result Group** to all named wells in the plate
- Click **Link the plate for Run** and enter Run Name
- Click **Start Run**

## 7. Analysis

For general instructions on automatic sample analysis, refer to the *GeneScan®* or *GeneMapper® ID or GeneMapper®ID-X Software User's Manual.*

**Note**: Within the Mentype® DIPscreen the red panel should be faded out.

Finding the exact lengths of the amplified products depends on the device type, the conditions of electrophoresis, as well as the DNA size standard used. The DNA Size Standard 550 (BTO) shall thus be used with the following lengths of fragments: **60**, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240**, **250, 260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550** bp.

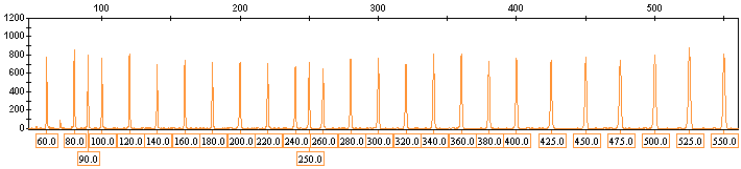



**Fig. 7** Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp

**Note:** The provided template files for the DNA size standard SST-BTO_60-450bp can be applied for the evaluation and analysis of the Mentype® **DIPscreen** using the GeneMapper® ID or ID-X Software.

## 7.1 Biotype® template files

Allele allocation should be carried out with suitable analysis software, e.g. the GeneMapper® ID/ID-X software in combination with the Mentype® DIPscreen template files from Biotype or the Chimeris™**Monitor** Software of Biotype Diagnostic GmbH. Template files are available from our homepage or as CD-ROM on request.

Recommended Biotype® templates for GeneMapper® ID/ID-X Software are:

| | | |
|---|---|---|
| Panels | DIPscreen_Panels_v0/v0X | or higher versions |
| BinSets | DIPscreen_Bins_v0/v0X | or higher versions |
| Size Standard | SST-BTO_60-450bp | |
| Analysis Method | Analysis_DIPscreen_310_200rfu | |
| | Analysis_DIPscreen_310_1000rfu | |
| | Analysis_DIPscreen_3130_200rfu | |
| | Analysis_DIPscreen_3130_1000rfu | |
| Plot Settings | PlotsBT5_4dyes | |
| Table Settings | Table for 2 alleles | |

Panels and BinSets always have to be used whereas the other template files are optional.
The prepared Biotype® templates for GeneMapper® ID/ID-X Software were generated for POP4® runs. In case of using other polymer types changes may necessary on Panels and Bins or within the Analysis Method before analyzing the data.
For detailed instruction please refer to the instruction Biotype® Template Files für GeneMapper® which can be downloaded from our homepage (www.biotype.de).

**Important Note:** Import and allele calling with provided template files is only guarantied using GeneMapper® ID/ID-X software. If GeneMapper® software is applied you may experience import problems using some template files. You may have to adjust Panels and Bins with one ore more runs of the allelic ladder on your specific instrument setup. Contact us for support (support@biotype,de).

### General procedure for the analysis

1. Check the DNA size standard
2. Check the allelic ladder
3. Check the positive control
4. Check the negative control
5. Analyse and interpret the sample data

## 7.2 Controls

The Control DNA XY13 of the test kit and other commercially available DNA from standard cell lines represent the following alleles:

**Table 2.** Allele determinations of Mentype® DIPscreen

| Locus | Control-DNA XY13 | ATCC K-562 | CCR 9947A | CCR 9948 | CCR 3657 |
|---|---|---|---|---|---|
| AM | XY | XX | XX | XY | XY |
| HLD106 | +/+ | -/- | +/+ | +/+ | +/+ |
| HLD70 | -/+ | -/+ | +/+ | -/+ | -/- |
| HLD84 | -/+ | +/+ | -/- | -/+ | -/- |
| HLD103 | +/+ | -/- | -/+ | +/+ | -/+ |
| HLD104 | -/+ | -/- | -/+ | +/+ | -/- |
| HLD116 | -/+ | +/+ | -/- | -/+ | -/- |
| HLD112 | -/+ | +/+ | -/+ | -/+ | -/+ |
| HLD307 | +/+ | +/+ | -/+ | +/+ | +/+ |
| HLD310 | +/+ | -/+ | -/+ | -/- | -/+ |
| HLD110 | -/+ | -/+ | -/+ | -/+ | -/+ |
| HLD133 | -/+ | -/- | +/+ | +/+ | -/+ |
| HLD79 | +/+ | +/+ | +/+ | -/+ | +/+ |
| HLD105 | -/+ | -/- | -/+ | -/+ | -/+ |
| HLD140 | +/+ | +/+ | -/- | -/+ | +/+ |
| HLD163 | +/+ | -/+ | -/+ | +/+ | -/+ |
| | | | | | |
| HLD91 | -/+ | -/+ | -/- | -/- | -/+ |
| HLD23 | -/+ | +/+ | -/- | -/+ | -/+ |
| HLD88 | +/+ | -/- | -/- | -/+ | +/+ |
| HLD101 | -/+ | -/+ | -/+ | -/+ | -/+ |
| HLD67 | -/+ | -/+ | +/+ | +/+ | +/+ |
| HLD301 | -/+ | -/+ | -/+ | -/+ | -/- |
| HLD53 | +/+ | -/- | -/+ | +/+ | -/- |
| HLD97 | -/- | -/- | -/+ | -/+ | +/+ |
| HLD152 | -/- | +/+ | +/+ | -/+ | +/+ |
| HLD128 | -/+ | -/+ | -/+ | -/- | -/+ |
| HLD134 | -/+ | -/- | +/+ | +/+ | -/- |
| HLD305 | -/+ | -/+ | -/+ | +/+ | -/+ |
| | | | | | |
| HLD48 | -/+ | +/+ | +/+ | -/+ | +/+ |
| HLD114 | +/+ | -/- | -/- | +/+ | -/+ |
| HLD304 | +/+ | -/- | -/+ | -/+ | -/- |
| HLD131 | +/+ | -/+ | -/- | -/+ | +/+ |
| HLD38 | +/+ | -/+ | -/+ | +/+ | +/+ |
| HLD82 | +/+ | +/+ | +/+ | -/+ | +/+ |

The reference DNA K-562 is available from ATCC (http://atcc.org/Produtcs/PurifiedDNA.cfm#celllines), DNA 9947A, 9948 and 3657 are available from Coriell Cell Repositories (CCR; http://locus.umdnj.edu/nigms/) .

### 7.3 Lengths of fragments and alleles

**Table 3** show the fragment lengths of individual alleles that refer to the DNA Size Standard 550 (BTO). All analyses have been performed on an ABI PRISM® 3130 Genetic Analyzer with POP4® polymer. Different analysis instruments, DNA size standards or polymers may result in different fragment lengths. In addition, a visual alignment with the allelic ladder is recommended.

### Scaling

Horizontal: 70-430bp (see Fig. 8 and 9)
Vertical: Depending on signal intensity

## Figure 8

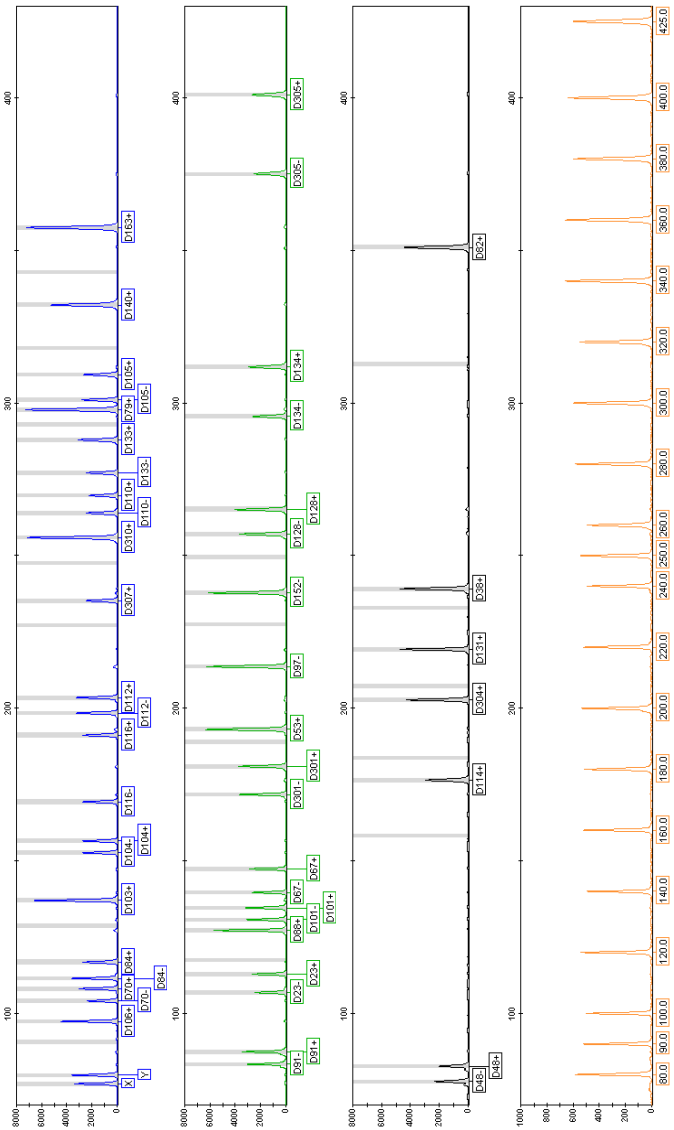



**Fig. 8** Electropherogram of the Mentype® **DIPscreen** using 1ng Control DNA XY13. Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (BTO). Allele assignment was performed using the GeneMapper® ID Software and the Mentype® **DIPscreen** template file.

## Figure 9

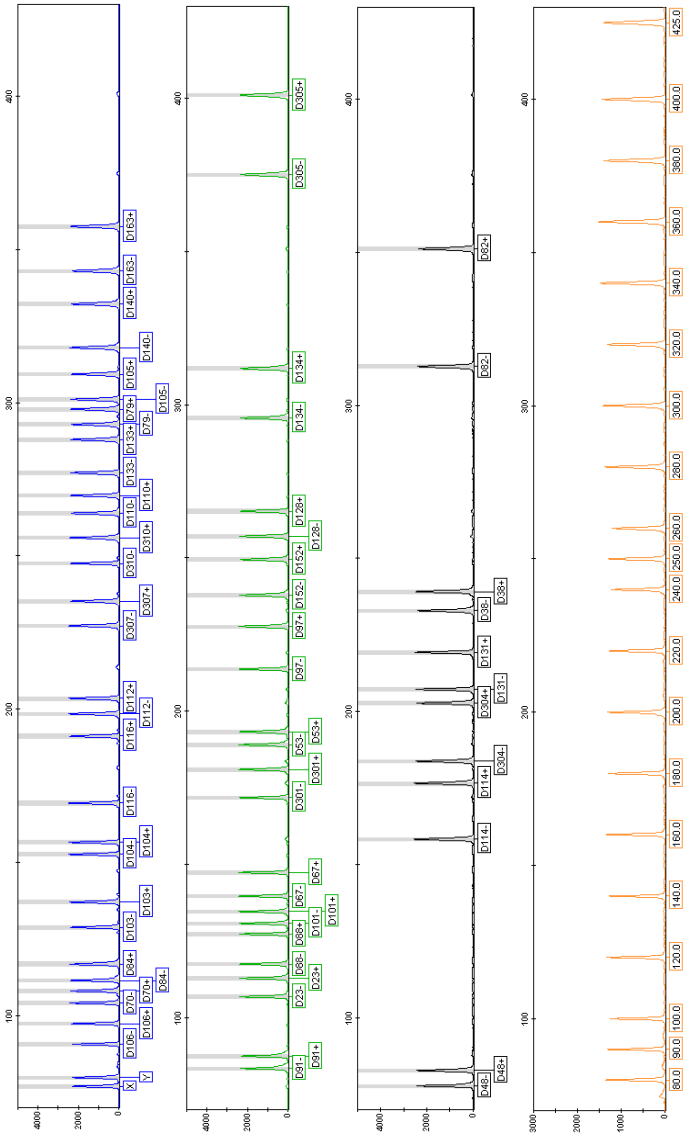



**Fig. 9** Electropherogram of the allelic ladder Mentype® **DIPscreen**. Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer with the DNA Size Standard 550 (BTO). Allele assignment was performed using the GeneMapper® ID Software and the Mentype® **DIPscreen** template file.

**Table 3.** Fragment lengths of the Mentype® **DIPscreen** allelic ladder analysed on an ABI PRISM® 3130 Genetic Analyzer with POP4® (FAM, BTG, BTY panel)

| Marker/FAM | -DIP [bp]* | +DIP [bp]* | Marker/BTG | -DIP [bp]* | +DIP [bp]* |
|---|---|---|---|---|---|
| AM | 77 (X) | 80 (Y) | HLD91 | 84 | 88 |
| HLD106 | 91 | 98 | HLD23 | 107 | 113 |
| HLD70 | 104 | 108 | HLD88 | 118 | 128 |
| HLD84 | 112 | 117 | HLD101 | 131 | 135 |
| HLD103 | 129 | 138 | HLD67 | 140 | 148 |
| HLD104 | 153 | 1157 | HLD301 | 172 | 182 |
| HLD116 | 170 | 192 | HLD53 | 190 | 194 |
| HLD112 | 199 | 204 | HLD97 | 214 | 228 |
| HLD307 | 228 | 236 | HLD152 | 239 | 250 |
| HLD310 | 248 | 257 | HLD128 | 258 | 266 |
| HLD110 | 264 | 270 | HLD134 | 296 | 312 |
| HLD133 | 278 | 288 | HLD305 | 375 | 401 |
| HLD79 | 294 | 299 | | | |
| HLD105 | 302 | 310 | **Marker/BTY** | **-DIP [bp]*** | **+DIP [bp]*** |
| HLD140 | 318 | 333 | HLD48 | 78 | 83 |
| HLD163 | 344 | 358 | HLD114 | 159 | 177 |
| | | | HLD304 | 184 | 203 |
| | | | HLD131 | 208 | 220 |
| | | | HLD38 | 234 | 240 |
| | | | HLD82 | 314 | 352 |

* rounded to integer

## 8. Interpretation of results

As mentioned above, post PCR analysis and automatic allele allocation with suitable analysis software ensure a precise and reliable discrimination of alleles.

The automated identification of informative DIP-loci directly from raw data of fragment analysis runs and the selection of suitable DIPquant assays for monitoring could be performed by using Chimeris™**Monitor** Software from Biotype Diagnostic GmbH.

### Pull-up peaks

Pull-up peaks may occur if peak heights are outside the linear detection range (>3000 RFU), or if an incorrect matrix was applied. They appear at positions of specific peaks in other colour channels, typically with lower signal intensities. Peak heights should not exceed 3000 RFU in order to prevent pull-up peaks.

### Template-independent addition of nucleotides

Because of its terminal transferase activity, the Multi Taq DNA Polymerase tends to add an adenosine radical at the 3'-end of the amplified DNA fragments. The artefact peak is one base shorter than expected (-1 bp peaks). All Biotype® primers are designed to minimise these artefacts. Artefact formation is further reduced by the final extension step of the PCR protocol at 68 °C for 60 min. Peak height of the artefact correlates with the amount of DNA. Laboratories should define their individual limits for analysis of the peaks.

### Artefacts

Room temperature may influence the performance of PCR products on multi-capillary instruments, shoulder peaks or split peaks occur. Furthermore, automated assignment could be influenced in some cases. If these effects occur we recommend injecting the sample again at higher room temperature and maybe using more than one allelic ladder sample per run.

### Influence of polymers

Mentype® **DIPscreen** was validated and certified for the analysis on POP4® polymer. The use of other polymers (e.g. POP7™ or POP6™) might influence the run behaviour of specific PCR products. Furthermore background noise might increase through different behaviour of free fluorescent dyes.

## 9. References

**Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C, Lamy T, Le Prise PY, Beauplet A, Bories D, Semana G, Quelvennec E. (2002)** Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. *Blood 99, 4618-4625*.

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**Qin XY, Li GX, Qin YZ, Wang Y, Wang FR, Liu DH, Xu LP, Chen H, Han W, Wang JZ, Zhang XH, Li JL, Li LD, Liu KY, Huang XJ (2011)** Quantitative assessment of hematopoietic chimerism by quantitative real-time polymerase chain reaction of sequence polymorphism systems after hematopoietic stem cell transplantation. *Chin Med J (Engl.) 124, 2301-2308*.

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**Wilhelm J, Reuter H, Tews B, Pingoud A, Hahn M (2002)** Detection and quantification of insertion/deletion variations by allele-specific real-time PCR: application for genotyping and chimerism analysis. *Biol Chem 383, 1423-1433*.

## 10. Explanation of Symbols

**Manufacturer**

**Date of manufacture**

**Batch code**

**Contains sufficient reagents for <N> tests**

**Consult instructions (handbook) for use**

**Use by**

**Temperature limitations**

**Catalogue number**

**In-Vitro-Diagnostics**

## Notes

## Notes